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PHYTOCHEMISTRY

β-Carboline alkaloids from *Hedyotis capitellata*

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Abstract

Three new β-carboline alkaloids were isolated from *Hedyotis capitellata* (Rubiaceae). Their structures were elucidated by spectroscopic data and X-ray analysis. © 1999 Elsevier Science Ltd. All rights reserved.

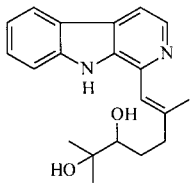
Keywords: *Hedyotis capitellata*; Rubiaceae; β-Carboline alkaloids; (−)-Isocyclocapitelline; (+)-Cyclocapitelline; Isochrysotricine

1. Introduction

The phytochemistry of plants belonging to the Rubiaceae family is mainly characterised by iridoids and indole alkaloids (Hegnauer, 1973, 1990). Plants of the genus *Hedyotis* have been widely used in the traditional Chinese and Vietnamese medicine (Do Tat Loi, 1991; Xin Hua Beng Cao Gang Yao, 1991). Recently, we reported the isolation and structural determination of a new β-carboline alkaloid, named capitelline, from *H. capitellata* (Phuong, Sung, Schmidt, Porzel & Adam, 1998). In continuation of this work, we now report the structural elucidation of three further alkaloids, named (−)-isocyclocapitelline, (+)-cyclocapitelline and isochrysotricine from the same plant species.

2. Results and discussion

After a routine work-up procedure of a methanol extract of *H. capitellata* followed by flash chromatography and repeated silica gel chromatography besides capitelline (**1**) (Phuong et al., 1998), the new alkaloids



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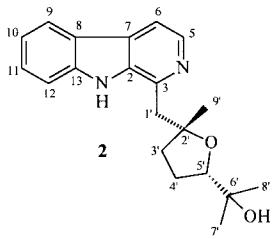

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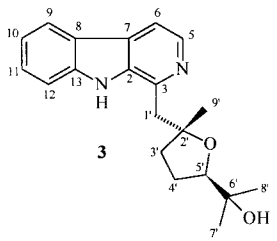

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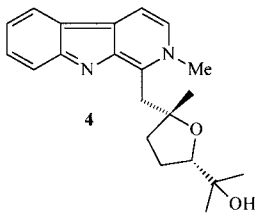

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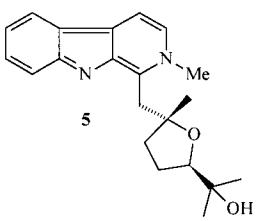

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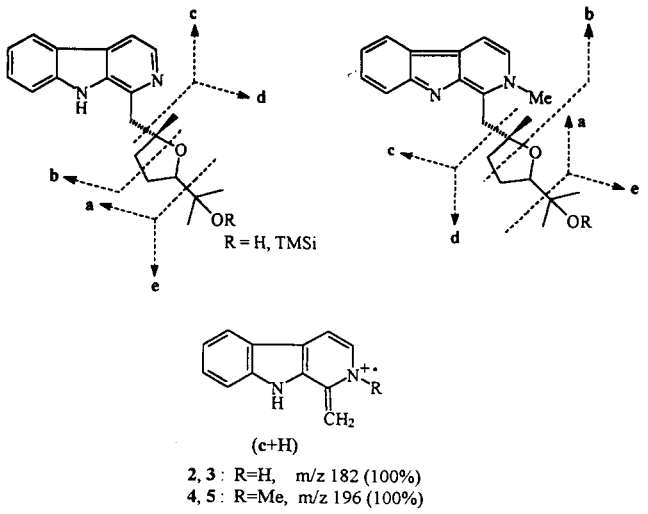

Scheme 1. Mass spectral fragmentation of compounds **2**–**5**.

(−)-isocyclocapitelline (**2**), (+)-cyclocapitelline (**3**), isochrysotricine (**4**) were obtained. Furthermore, the alkaloid chrysotricine (**5**), recently found in *H. chrysotricha* (Peng, Feng, Zheng & Liang, 1997) was isolated. The UV spectra of compounds **2**–**4** are characteristic for β-carboline alkaloids. The molecular formula of compounds **2** and **3** were assigned to be $C_{20}H_{24}N_2O_2$ by HR-EIMS. Their mass spectral fragmentation is mainly characterised by α-cleavages of the monoterpenoid moiety leading to the complementary key ions **a** and **e** as well as **c** and **d**, respectively (Scheme 1). Ion **b** is originated by cleavage through the furane ring. The ion of type (**c**+H) at *m/z* 182 ($C_{12}H_{10}N_2$) represents the basic harman skeleton (Peng et al., 1997; Phuong et al., 1998). In the mass spectra of the trimethylsilyl derivatives of **2** and **3** obtained by GC–MS the key ions **d** and **e** show the corresponding mass shift of 72 amu. The mass spectral behaviour of compounds **4** and **5** is very similar to that of **2** and **3**. The ions of

Table 2
^{13}C chemical shifts of compounds **2**–**5**

C	**2**[a]	**3**[a]	**4**[b]	**4·HCl**[c]	**5**[b]	**5·HCl**[c]
2	135.9	135.8	147.1	137.8	147.5	137.9
3	142.7	142.9	143.2	142.3	143.5	142.2
5	137.5	138.0	128.2	136.0	128.1	136.5
6	113.2	113.5	115.1	117.1	115.2	117.2
7	128.8	129.0	132.6	133.7	132.5	133.8
8	121.7	121.8	123.0	121.1	123.2	121.2
9	121.6	121.7	123.0	124.2	123.1	124.3
10	119.6	119.7	118.8	122.9	118.7	123.2
11	128.0	128.2	129.3	133.0	129.3	133.2
12	111.7	111.7	118.7	114.0	118.9	113.8
13	140.8	140.7	157.6	145.8	158.0	145.6
NMe			45.8	46.6	45.7	46.9
1′	46.6	48.0	86.0	41.3	86.5	40.8
2′	84.0	85.1	86.0	85.7	86.4	86.0
3′	39.0	36.8	37.9 br	39.6	38.3 br	38.5
4′	26.1	26.1	26.6	26.9	27.2	27.3
5′	86.3	86.8	86.4	87.8	88.3	88.6
6′	71.3	70.3	72.1	71.8	71.7	71.6
7′[d]	27.8	28.0	26.2	26.5	26.2	26.4
8′[d]	25.8	24.0	25.5	27.0	25.4	25.1
9′	25.8	27.4	26.8 br	26.2	29.2 br	28.3

[a] Solvent $CDCl_3$.
[b] Solvent $CD_3OD + K_2CO_3$ (pH 9).
[c] Solvent CD_3OD.
[d] Diastereotopic methyl groups are not assigned.

Table 1
^{1}H NMR data of compounds **2**–**5** (values in bold face are chemical shifts of HSQC correlation peaks) (n.d. means not detected because of broad signals or H/D exchange)

H	**2**[a]	**3**[a]	**4**[b]	**4·HCl**[c]	**5**[b]	**5·HCl**[c]
5	8.35 d (5.3)	8.37 d (5.4)	7.88 d	8.48 d (6.5)	7.90 d	8.52 s
6	7.84 d (5.3)	7.87 d (5.4)	8.15 d	8.50 d (6.5)	8.17 d	8.52 s
9	8.10 d (7.9)	8.12 d (7.9)	8.16 d	8.36 d (8.1)	8.17 d	8.38 d (8.1)
10	**7.25**	7.27 dd (7.9/7.3)	7.10 dd	7.44 dd (8.1/6.8)	7.09 dd	7.46 dd (8.1/6.6)
11	**7.51**	7.52 dd (8.2/7.3)	7.48 dd	7.77 dd (8.2/6.8)	7.47 dd	7.79 dd (8.2/6.6)
12	**7.51**	7.45 d (8.2)	7.76 d	7.81 d (8.2)	7.74 d	7.76 d (8.2)
NH	9.91 s	9.84 s	n.d.		n.d.	
NMe			4.46 s	4.53 s	4.51 s	4.59 s
1′	3.49 d (13.7)/3.22 d (13.7)	3.54 d (14.4)/3.42 d (14.4)	n.d.	3.80 s	n.d.	3.86 d (15.1)/3.83 d (15.1)
3′	**2.10**/**1.95**	**2.02**/**1.95**	**2.08**/**1.90**	**2.12**	**2.23**/**1.94**	2.13 dd (11.8/7.3)/1.94 m
4′	**2.10**/**1.98**	**1.95**/**1.74**	**1.85**/**1.57**	**2.05**	**1.72**/**1.59**	1.85 m/1.72 m
5′	3.91 m	3.59 dd (10.0/5.7)	3.78 dd	3.89 dd (8.1/6.6)	**3.86**	3.44 dd (10.2/5.4)
7′[d]	1.31 s	1.32 s	0.97 s	1.17 s	1.11 s	1.12 s
8′[d]	1.20 s	1.11 s	0.81 s	1.08 s	1.06 s	1.07 s
9′	1.25 d (0.6)	1.28 s	n.d.	1.32 s	n.d.	1.36 s

[a] Solvent $CDCl_3$.
[b] Solvent $CD_3OD + K_2CO_3$ (pH 9).
[c] Solvent CD_3OD.
[d] Diastereotopic methyl groups are not assigned.

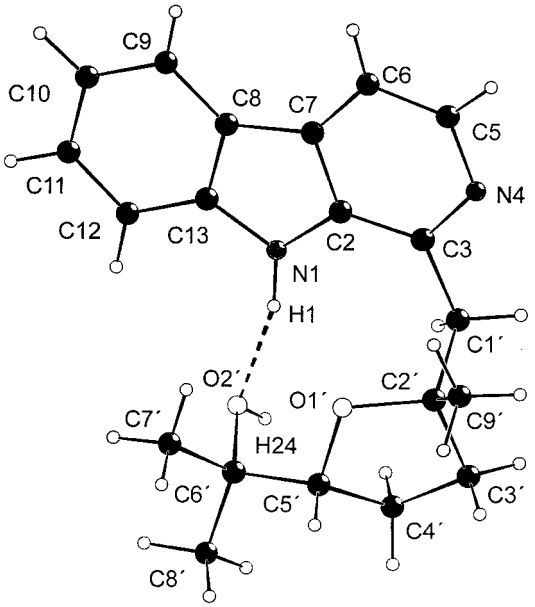

Fig. 1. Molecular structure of (−)-isocyclocapitelline (**2**) (hydrogen atoms are omitted).

type **a** and (**c**+H) are shifted by 14 amu to *m*/*z* 237 and 196, respectively, indicating an additional methyl substitution at the harmane skeleton (Scheme 1).

Compounds **2** and **3** show quite similar ^{1}H and ^{13}C NMR spectra with only slightly different chemical shifts of the side chain atoms 1′–9′. As a result of combined use of one- and two-dimensional NMR experiments (including APT, COSY, NOESY, GHSQC, GHMBC) the same constitution is found for both compounds **2** and **3**, which thus have to be stereoisomers. A weak NOESY cross peak between H-5′ and Me-9′ in **2**, which is not found in the NOESY spectrum of **3**, suggests a *cis*-orientation of H-5′ and Me-9′ in **2**, but a *trans*-orientation in **3**. The ^{1}H and ^{13}C NMR data of **2** and **3** are given in Tables 1 and 2.

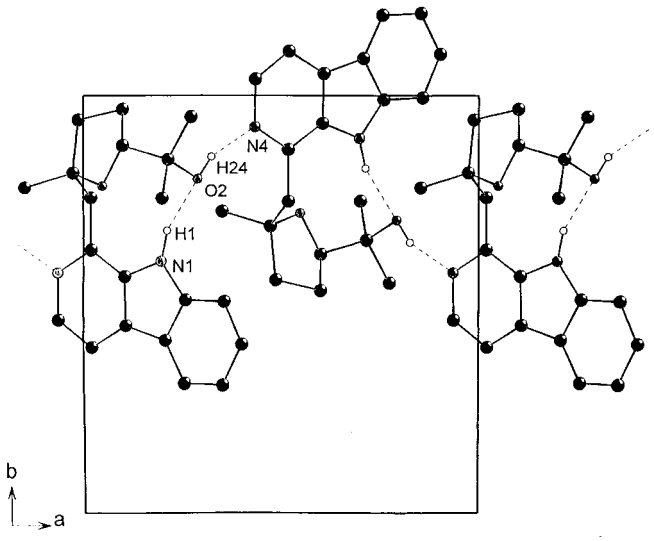

Fig. 2. Molecular arrangement of (−)-isocyclocapitelline (**2**) in the crystal (hydrogen bonds represented by dashed lines).

The constitution and relative configuration of (−)-isocyclocapitelline (**2**) was confirmed by X-ray analysis. The obtained molecular structure is shown in Fig. 1. Due to the lack of any heavier elements the absolute configuration could not be determined. The bond lengths and angles are in the expected range. The most important feature of the molecular structure of **2** is an intramolecular hydrogen bond between the atoms N(1) and O(2′) (N(1)–H(1): 94(2) pm, H(1)···O(2′): 192(2) pm). A second intermolecular hydrogen bridge is observed between the hydroxy proton H(24) and the nitrogen atom N(4) of a neighbouring molecule (O(2′)–H(24): 96(2) pm, H(24)···N(4): 191(3) pm. As a consequence of these intermolecular hydrogen bridges the molecules are arranged in a one-dimensional chain in the direction of the crystallographic *a*-axis (Fig. 2).

In the ^{1}H NMR spectrum of **4** an additional methyl group signal is found, which has to be assigned to a N-methyl group because of its ^{1}H (δ 4.53) and ^{13}C (δ 46.6) chemical shift. The HMBC correlations, which are found between the proton signal of the N-Me group and C-3 and C-5, prove the position of the methyl group at N-4. The complete structural elucidation of **4** is done by means of detailed NMR investigations, especially ^{1}H,^{1}H COSY, GHSQC and GHMBC experiments. The multitude of connectivities found in the 2D spectra allows an unequivocal assignment of all ^{1}H and ^{13}C NMR signals (Tables 1 and 2) with the exception of H1′ and Me9′. The structure stated for **4** is beyond it in full agreement with the MS data of this compound. Compound **5** shows similar ^{1}H and ^{13}C chemical shifts in comparison with **4**. The 2D NMR correlation peak patterns of **5** are identical with that of **4** with the exception of a NOE correlation between H5′ and Me9′ (detectable for **4·HCl**), which could be observed only in case of **4**. The ^{1}H chemical shifts of **4** and **5** were strongly influenced by the solvent and pH. Therefore, K_2CO_3 was added to the solution in CD_3OD until a pH of 9. Under these conditions, apparently a tautomeric transformation occurred as described for chrysotricine (**5**) which was recently isolated by Peng et al. (1997). The ^{1}H NMR signals of the protons attached to C1′ and C9′ could not be detected presumably because they are very broad as a result of the tautomeric equilibrium. The ^{1}H and ^{13}C NMR data of the side chain atoms 1′-9′ of **5** are in good agreement with those of chrysotricine measured in $CDCl_3$ solution (Peng et al., 1997). However, for the NMR signals of the harman skeleton atoms 1–13 in CD_3OD solution with pH ca. 7 partly big differences occur in chemical shifts of chrysotricine and **5**. However, after adding K_2CO_3 to the solution of **5** in CD_3OD until a pH of 9, a good agreement of

the ^{13}C chemical shifts with those of chrysotricine were observed. This can only be explained by transformation of **5** into the hydrochloride in the course of isolation. The same is true for **4**.

The new β-indole alkaloids **2** and **3** can be biosynthetically formed by a ring closure of capitelline via C-2′ and the hydroxy function at C-5′ or as discussed for **5** as products of coupling a tryptamine moiety with a linaoyl oxide unit (Peng et al., 1997).

3. Experimental

3.1. General methods

Mps. uncorr.; $[\alpha]_D$: Jasco DIP 1000 polarimeter; NMR (Varian Unity 500): 499.85 (^1H-) and 125.7 (^{13}C), solvent $CDCl_3$, TMS as int. standard; MS (AMD 402, AMD Intectra GmbH): 70 eV EIMS (DIS), HR-EIMS (resolution 7.500); IR (Bruker IFS 28): KBr discs; GC–MS (MD-800, Fisons Instruments): 70 eV EI, source temp. 200°C, column DB-5MS (J&W, 15 m × 0.32 mm, 0.25 μm film thickness), inj. temp. 260°C, interface temp. 300°, carrier gas He, flow rate 1 ml/min, splitless injection, column temp. program: 170° for 1 min, then raised to 290°C at a rate of 30°/min and held on this temperature for 20 min. The relative retention times (RRT) were calculated with respect to squalene (RT = 17.87 min). The trimethylsilylation of (−)-isocapitelline (**2**) and (+)-cyclocapitelline (**3**) was carried out by *N*,*O*-bistrimethylsilylacetamide.

3.2. Plant material

H. capitellata was collected in May 1996 in the National Park Cuc Phuong, Province of Ninh Binh, Vietnam. The species was identified by Dr. Tran Dinh Dai, Hanoi. A Voucher specimen (No. 2049) is deposited in the Herbarium of the Institute of Ecology and Natural Resources of the National Centre for Scientific Research, Hanoi, Vietnam.

3.3. Extraction and purification of the alkaloids

The aerial part of the dried plants of *H. capitellata* (1.3 kg) were powdered and extracted 4× with 95% MeOH at room temp. After evaporating the MeOH the aqueous layer was acidified with 0.5 N HCl, and then extracted with diethyl ether–toluene (1:1 v/v). The aqueous layer was basified with K_2CO_3 (pH 9–10) and then extracted with $CHCl_3$/EtOH (2:1 v/v). The solid residue (7 g) showed positive reaction with Dragendorff reagent and a fluorescence at 254 nm.

The residue was flash-chromatographed (silica gel, Merck 60, 0.04–0 063 mesh) with acetone/*n*-hexane (1:1 v/v), acetone/*n*–hexane (3:1 v/v), acetone and $CHCl_3$/MeOH/H_2O (14:5:1 v/v) as eluent system. The elution was carried out stepwise in 50 ml frs. Fr. 3 (290 mg) was rechromatographed by prep. TLC (Merck 60, 1 mm) using acetone/*n*-hexane (1:1) as eluent yielding (+)-cyclocapitelline (**3**).

The combined frs 6 and 7 (297 mg) were further purified by repeated flash chromatography with EtOAc/*n*-hexane (7:3 v/v) as eluents to give (−)-isocyclocapitelline (**2**).

3.4. (−)-Isocyclocapitelline (2)

15 mg, yellow prisms, m.p. 199–200°C (acetone), $[\alpha]_D$ −75° ($CHCl_3$, *c* 0.50); EIMS *m*/*z* (rel. int.): 324.1830 (M^+, 10, calc. for $C_{20}H_{24}N_2O_2$ 324.1837), 309 (5), 265.1317 (**a**, 19, calc. for $C_{17}H_{17}N_2O$ 265.1341), 223 ([**b** + H], 26), 221 ([**b**-H], 7), 207 (5), 206 (6), 182.0828 ([**c** + H], 100, calc. for $C_{12}H_{10}N_2$ 182.0844), 154 (6), 143 (**d**, 21), 125 ([**d**-H_2O], 18), 107 (6), 97 (5), 85 (11), 71 (18), 59 (**e**, 6); UV (EtOH) λ_{max} nm (log ϵ): 242 (4.36), 290 (4.14), 339 (3.62); IR cm^{-1} ($CHCl_3$): 3292 (OH), 2972, 1627, 1568, 1475, 1456, 1379, 1324; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.5. (−)-Isocyclocapitelline-trimethylsilylether

RRT = 0.98, EIMS *m*/*z* (rel. int.): 396 (M^+, 10), 381 (11), 265 (**a**, 30), 235 (9), 223 ([**b** + H], 56), 221 ([**b**-H], 24), 215 (**d**, 38), 206 (20), 182 ([**c** + H], 100), 157 (20), 143 (30), 131 (**e**, 72), 125 (24), 73 (73).

Crystal data of **2**. $C_{20}H_{24}N_2O_2$, M = 324,41, MoK_α (λ = 71,073 pm), orthorhombic, space group P $2_12_12_1$, with a = 11.449(3) Å, b = 11.938(3) Å, c = 13.146 (4) Å, Z = 4, V = 1796.8 (8) Å^3, D_c = 1.199 g/cm^3, $\mu(MoK_\alpha)$ = 0.078 mm^{-1}, $F(000)$ = 696, Θ range for data collection 2.36–26°, index ranges $-13 \le h \le 14$, $-13 \le k \le 14$, $-16 \le l \le 16$, 11057 collected reflections, 2329 independent reflections with $I > 2\sigma(I)$. The structure was solved by direct methods (SHELXS 86) (Sheldrick, 1986, 1993) with 344 refined parameters, final R indices: $R1$ ($I > 2\sigma(I)$) = 0,0338, $wR2$ (all data) = 0,0589, largest difference peak and hole 0,119 and −0.081 eÅ^{-3}. The data were collected on a STOE IPDS. The structure plots were created with Diamond (Bergerhoff & Brandenburg, 1996). The atomic coordinates for this work are available on request from the director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this paper.

3.6. (+)-Cyclocapitelline (3)

28 mg, yellow amorphous solids, $[\alpha]_D$ +43° ($CHCl_3$,

c 0.50); EIMS *m/z* (rel. int.): 324 (M^+, 17), 309 (9), 265 (**a**, 29), 223 ([**b**+H], 15), 221 ([**b**-H], 10), 207 (6), 206 (6), 182 ([**c**+H], 100), 154 (6), 143 (**d**, 41), 125 ([**d**-H_2O], 28), 107 (6), 97 (4), 85 (15), 71 (22), 59 (**e**, 5); UV (EtOH) λ_{max} nm ($\log \epsilon$): 242 (4.30), 289 (4.07), 338 (3.59); IR cm^{-1} ($CHCl_3$): 3342 (OH), 2975, 1626, 1494, 1455, 1379, 1325; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

3.7. (+)-Cyclocapitelline-trimethylsilylether

RRT = 0.97 min, EIMS *m/z* (rel. int.): 396 (M^+, 1), 381 (4), 265 (**a**, 18), 235 (4), 223 ([**b**+H], 23), 221 ([**b**-H], 11), 215 (**d**, 19), 206 (9), 182 ([**c**+H], 100), 157 (12), 143 (18), 131 (**e**, 40), 125 (16), 73 (71).

The combined frs 15 and 16 (287 mg) contained the main alkaloid, identified recently as capitelline (**1**) (Phuong et al., 1998).

The combined frs 196–207 (100 mg) were rechromatographed by prep. TLC using $CHCl_3$/MeOH/H_2O (14:5:1 v/v) as eluent yielding (−)-isochrysotricine (**4·HCl**). Frs 223–229 (79 mg) were further purified in the same way to give chrysotricine (**5·HCl**).

3.8. Isochrysotricine HCl (*4·HCl*)

22 mg, yellow amorphous solids, $[\alpha]_D$ −110° (MeOH, *c* 0.50); EIMS *m/z* (rel. int.): 338.1979 (M^+, 5, calc. for $C_{21}H_{26}N_2O_2$ 338.1994), 323 (3), 320 ([M-H_2O]$^+$, 4), 279 (**a**, 11), 237.1388 ([**b**+H], 32, calc. for $C_{16}H_{17}N_2$ 237.1392), 221 (6), 207 (3), 206 (3), 196.1010 ([**c**+H], 100, calc. for $C_{13}H_{12}N_2$ 196.1000), 182 (5), 181 (4), 168 (4), 154 (5), 143 (**d**, 3), 125 ([**d**-H_2O], 4), 85 (4), 71 (8), 59 (**e**, 5); UV (EtOH) λ_{max} nm ($\log \epsilon$): 210 (4.13), 250 (4.05), 311 (3.95); IR cm^{-1} ($CHCl_3$): 3373 (OH), 2926, 1633, 1576, 1457, 1383, 1258, 1058, 788, 757; ^1H NMR: Table 1; ^{13}C-NMR: Table 2.

3.9. Chrysotricine HCl (*5·HCl*)

34 mg, yellow amorphous solids, $[\alpha]_D$ +10° (MeOH, *c* 0.50); EIMS *m/z* (rel. int.): 338.1981 (M^+, 12, calc. for $C_{21}H_{26}N_2O_2$ 338.1994), 323 (5), 320 ([M-H_2O]$^+$, 8), 279 (**a**, 13), 237.1401 ([**b**+H], 46, calc. for $C_{16}H_{17}N_2$ 237.1392), 221 (15), 207 (8), 206 (8), 196.1015 ([**c**+H], 100, calc. for $C_{13}H_{12}N_2$ 196.1000), 181 (12), 168 (14), 154 (16), 143 (**d**, 7), 127 (8), 125 ([**d**-H_2O], 7), 85 (12), 71 (19), 59 (**e**, 27); UV (EtOH) λ_{max} nm ($\log \epsilon$): 210 (4.05), 249 (4.02), 310 (3.94); IR cm^{-1} ($CHCl_3$): 3405 (OH), 2927, 1633, 1449, 1337, 1156, 1053, 757; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

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